

QUALITATIVE STUDIES OF PYROLYSIS OF DIPHENYLAMINE, CARBAZOLE, ACRIDINE AND NICOTINAMIDE AT 1050° C

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Products from the high-temperature (1050°C) pyrolysis of diphenylamine, carbazole, acridine and nicotinamide were identified by chromatographic (GLC, TLC) and spectral (UV) techniques. Diphenylamine yielded predominantly carbazole, and nicotinamide generated benzonitrile as the major product. Other minor products were: 4-methylpyridine, quinoline, nicotinonitrile, indole derivatives, aniline and others. Acridine and carbazole were extremely stable, even at this temperature, and underwent only minor changes.

There have been many studies dealing with the presence and origin of biologically active constituents in cigarette smoke, especially of the polynuclear aromatic hydrocarbons (1,9). In contrast, there have been few reports (5,11) of similar studies dealing with polycyclic compounds containing nitrogen even though a number of these have been reported in cigarette smoke including several with biological activity (2,9,12,15). The presence of such compounds in cigarette smoke is not unexpected inasmuch as tobacco leaf contains likely precursors such as nicotine, proteins, amino acids and pigment. Studies in which the latter materials were pyrolyzed have previously been undertaken (3,4a,6,7,11,14).

In the present instance, we undertook a study of the pyrolysis of diphenylamine, carbazole, acridine¹, and nicotinamide, all of which occur to some extent in smoke (11), and one of which, nicotinamide, is present in leaf (11) as well. Although we were unable to observe the pyrosynthesis of biologically active nitrogenous heterocyclics, the other compounds formed proved to be similar, if not identical, to those present in tobacco smoke. The data presented below should therefore shed some light on their mode of formation at high temperatures

EXPERIMENTAL

Materials pyrolyzed. Materials pyrolyzed were obtained from commercial sources and were recrystallized before use; they were judged pure on the basis of their ultraviolet absorption and gas chromatographic characteristics.

Pyrolyses. Samples in 1.0 to 2.5 g quantities (in ceramic boats) were pyrolyzed in Mullite² or vycor tubes (5.10 cm x 1.9 cm o.d.) positioned horizontally and flushed continually by a stream of nitrogen (30 ml/min). Generally, the tube was packed with quartz chips. Tube temperature was maintained at the required level by a Lindberg Hevi-Duty Furnace which enclosed the tube. Because of the extreme thermal stability of at least three of the compounds (diphenylamine, carbazole, acridine) pyrolyzed, pyrolyses were generally performed

at 1050°C, rather than 872-884°C, the approximate burn temperature of a cigarette (11a). Pyrolysis conditions are summarized in Table 1. Residence times varied from 2.0 to 30.0 seconds, and were estimated by measuring the interval between introduction of the sample and the first appearance of "smoke" traversing the traps. The pyrolysis was considered complete when no more "smoke" was seen in the trapping system. Products were collected in a series of traps immersed in ice-water and dry ice-acetone. After exiting from the traps, the product stream passed into a gas bubbler containing 100 ml 5% aqueous HCl and 105 ml diethyl ether.

Fractionation of pyrolyzates. The pyrolyzates were washed out of the traps with 5% HCl (aqueous) and then with ether. The washings were mutually extracted and were then combined with the corresponding solutions in the gas bubbler.

The ether extract, containing neutral compounds, was dried over anhydrous sodium sulfate and carefully evaporated under a stream of nitrogen to 1 ml. The aqueous acidic solution was adjusted to a pH 12, with pellets of sodium hydroxide, in order to liberate bases which were then extracted with ether. The latter solution was also dried and concentrated to a volume of 1 ml. The resulting two ether solutions of neutrals and bases respectively were suitable for subsequent chromatographic and special analysis.

Gas chromatographic analysis. Gas chromatography was carried out initially with a Beckman GC-4 instrument under the following conditions (for both the neutral and basic products):

Columns. 6 ft x 0.125 in o.d. stainless steel columns packed with 3% SE-30 on 60/80 mesh chromosorb W.

Column temperature. 110°C for 1.6 min. and then programmed to 235°C at 8°C/min.

Inlet temperature. 250°C.

Detector temperature. 305°C.

Helium flow rate. 20 ml/min.

Detection. flame ionization.

In addition, columns (6 ft x 0.125 in o.d.) containing 10% carbowax 20 M on 60/80 mesh chromosorb W were also utilized for screening product mixtures on the above instrument. For this purpose, operating conditions were the same as above except that column temperature was

Table 1—Pyrolytic conditions

Compound pyrolyzed	Amount		Residence Time for complete	
	Pyrolyzed (g)	Temp. (°C)	Time (sec)	pyrolysis (min)
Diphenylamine	2.5	800	22.9	90
Diphenylamine	1.7	1050	7.9	7
Carbazole	1.5	1050	6.4	90
Acridine	1.25	1050	2.0	<1
Nicotinamide	1.0	1050	30	10

Table 2—Products^a from pyrolysis

	Diphenylamine of		Carbazole 1050°	Acridine 1050°	Nicotinamide 1050°
	800°	1050°			
Benzene	+		+	+	+
Pyridine	+		+	+	+
4-Methylpyridine	+	+	+		
Aniline	+	+			
Benzonitrile					+ ^b
Nicotinonitrile					+
Naphthalene				+	
1-Methylindole	+		+		
Quinoline	+	+			+
Indole	+	+			+
3-Methylindole		+			
2-Methylindole		+	+		
2,3-Dimethylindole	+	+	+	+	
Diphenylamine	+	+	+		
Acridine	+	+	+	+	
Carbazole	+ ^b	+ ^b	+ ^b	+	
2-Methylcarbazole	+	+	+		
Phenanthridine	+	+			

^a In order of GLC retention times. Identifications are tentative.

^b Major products.

programmed to 225°C (from 110°C) at 7°C/min. Retention data for comparative purposes were obtained by injecting 1 μ l quantities of standard ether solutions (1%) of known substances into the above instrument.

Collection of gas chromatographic peak effluents for spectral analysis was achieved with a Varian-Aerograph gas chromatograph used under the following conditions for both neutrals and bases:

Columns. 10 ft x 0.25 in o.d. stainless steel columns packed with 15% SE-52 on 60/80 chromosorb W.

Column temperature. Programmed from 70°C at 8°C/min

Injector temperature. 235°C

Detector temperature. 250°C

Helium flow rate. 86 ml/min

Detection. Thermal conductivity (150 ma.).

For "peak" collection, 100-200 μ l of solution (product mixture) were injected into the instrument equipped with hot wire detectors. As effluents exited from the instrument, they were trapped in U-shaped glass tubes cooled in dry ice-acetone.

They were then washed out of the tubes with appropriate solvents for analysis by ultraviolet absorption spectroscopy.

Thin-layer chromatographic analysis. Thin-layer chromatography was used as an auxiliary method for determining the composition of the pyrolyzates. A slurry, consisting of 70 g silica gel G (Merck) and 140 ml water, was applied to glass plates as a layer 0.5 mm thick. Plates were dried for 20 min at 110°C, and then stored in a desiccator until needed. Samples were either streaked or spotted on the plate prior to solvent development. Solvent systems used included: chloroform—96% acetic acid (95 mls + 5 mls) (10) for indole derivatives, and benzene-methanol (95 mls + 5 mls) (8) for nitrogen bases.

Detecting solutions for indoles generally were the van Urk reagent (4-dimethylaminobenzaldehyde in hydrochloric acid followed by aqua regia), SbCl₅ in CCl₄, and in some cases iodine in chloroform. Reagent solutions were applied to the plates as a spray (13).

Ultraviolet absorption spectroscopy. Absorption spectra were obtained with a Perkin-Elmer Model 202 Instrument. Solvent generally used was absolute methanol.

RESULTS AND DISCUSSION

Identification of products (Table 2). Compounds present in pyrolyzates were tentatively identified by co-gas chromatography, thin-layer chromatography and ultraviolet spectroscopy used interdependently, e.g. a band scraped from a TLC plate of resolved pyrolyzate was

eluted from the adsorbent with methanol and tentatively identified on the basis of its ultraviolet absorption spectrum and its retention characteristics on gas chromatographic columns. These procedures were generally followed except in those cases where the products were fairly volatile and subject to loss in fractionation and concentration steps. Thus, the presence of benzene, pyridine, 4-methylpyridine, aniline and quinoline was indicated in pyrolyzates on the basis of the UV absorption characteristics of individual fractions. A number of products from the pyrolysis of nicotinamide were additionally identified on the basis of the UV absorption spectra of their corresponding gas chromatographic peak effluents (benzonitrile, nicotinonitrile, naphthalene, indole, and 3-methylindole).

Diphenylamine. At 800°C, the major product from pyrolysis of diphenylamine was carbazole. Formation of carbazole which involves a simple cyclization of the parent compound has been previously reported (4); on the other hand, the presence of benzene and aniline in the diphenylamine pyrolyzate would suggest a rupture of the C-N bond in the starting material. The formation of other products is also difficult to rationalize; these include pyridine and 4-methylpyridine, a number of substituted indoles, acridine and phenanthridine. Similar products were obtained by pyrolyzing diphenylamine at 1050°C. However, based on gas chromatographic peak areas, there were no major products, and a number of products were absent. We noted less carbazole and more indoles and acridine in the pyrolyzate. In view of the temperature effects, one may speculate that the pathway from diphenylamine to substituted indoles proceeds through a carbazole intermediate. However, experiments in which carbazole was pyrolyzed at 1050°C demonstrated its marked stability (*vide infra*), although a number of indoles were found in the resulting pyrolyzate.

Carbazole. Pyrolysis of carbazole at 1050°C resulted in the formation of a number of new compounds (benzene, indoles, acridine, diphenylamine and others) in addition to the recovery of a large amount of starting material. The recovery of the starting material which constituted the major component of the product mixture seemed to preclude the role of carbazole as a principal intermediate in the "diphenylamine to substituted indole" pathway. Unsubstituted indole was not observed among the products from pyrolysis of carbazole.

Acridine. This compound, like carbazole, is extremely stable, as demonstrated by its pyrolytic behavior. It constituted the major portion of the pyrolyzate. Other minor constituents were benzene, pyridine, naphthalene, 2, 3-dimethylindole and carbazole. The presence of these compounds would suggest formation of intermediate materials involving homo- and heterolytic mechanisms.

Nicotinamide. Nicotinamide was pyrolyzed to determine the effect of the amide side chain on the composition of the product mixture. Heterocyclic acids, for example, have been reported to undergo rapid decarboxylation on pyrolysis (4). Nicotinamide underwent pyrolysis with the evolution of thick black smoke and the production of an odor attributable to hydrogen cyanide. We expected that in the course of the reaction the amide side chain would undergo loss of a molecule of water with the resultant formation of nicotinonitrile. Although the latter compound was produced, the major product was benzonitrile, the formation of which would necessitate rupture of the pyridine ring of nicotinamide.

Other components of the nicotinamide pyrolyzate included benzene pyridine, naphthalene, quinoline, indole and 3-methylindole.

CONCLUSIONS

In undertaking this study, we were trying to learn something of the pyrosynthesis of nitrogenous compounds which could influence the chemical and biological characteristics of tobacco smoke. Just as polynuclear aromatic hydrocarbons, and simple phenols are common to pyrolyzates of a variety of substances, so too are characteristic nitrogen-containing compounds common to pyrolyzates of other materials. Nicotine (11), proteins and amino acids (3) generate pyridines, quinoline, nitriles and indoles on pyrolysis, and as this study demonstrates so do other nitrogenous substances. Of significant interest is possible generation of benzcarbazoles and benzacridines on pyrolysis; however, improved procedures for the isolation and detection of these compounds are required for work along this line.

SUMMARY

Products from the high-temperature (1050°C) pyrolysis of diphenylamine, carbazole, acridine and nicotinamide were identified by chromatographic (GLC, TLC) and spectral (UV) techniques. Diphenylamine yielded predominantly carbazole, and nicotinamide generated benzonitrile as the major product. Other minor products were: 4-methylpyridine, quinoline, nicotinonitrile, indole derivatives, aniline and others. Acridine and carbazole were extremely stable, even at this temperature, and underwent only minor changes.

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